

校编码: 10384

分类号_____密级_____

学号: 24520101153286

UDC_____

厦 门 大 学

硕 士 学 位 论 文

基质金属蛋白酶 16 在食管鳞癌中的表达及其生物学功能

Expression and biological role of Matrix metalloproteinases
16 in esophagus squamous cell carcinoma

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论文提交日期: 2013 年 月

论文答辩时间: 2013 年 月

学位授予日期: 2013 年 月

答辩委员会主席: _____

评 阅 人: _____

2013 年 6 月

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摘要

背景及目的

食管癌是世界第六大癌症死因，临床上多数患者就诊时已经进入中晚期，手术效果差，五年生存率仅徘徊在 10%~20%。其治疗效果不佳的主要原因是肿瘤的侵袭和转移。基质金属蛋白酶是肿瘤侵袭和转移过程中周围基质降解和重塑得重要酶,但 MMP 抑制剂在多种癌症患者身上进行实验时,这种药物无法减缓肿瘤生长，有时甚至加快肿瘤生长，这提示并非所有 MMP 均为原癌基因。基质金属蛋白酶-16 在多种肿瘤组织中有较高的表达，但在食管癌中的表达及与食管癌侵袭和转移关系的研究目前尚未有报道。我们检测食管癌组织标本 MMP-16 的表达水平与临床病理资料的相关性，并采取 RNA 干扰的方法，建立了有效的 MMP-16 基因沉默的 Eca109 细胞株，观察食管鳞癌细胞在迁移和侵袭方面的改变，研究食管鳞癌治疗的策略以及探讨食管癌的治疗的基因靶点。

方法

1. 应用免疫组化、Western blot 及 Real Time PCR 检测食管癌及配对远癌组织的 MMP-16 蛋白及 MMP-16 mRNA 表达情况及临床意义。
2. 对食管鳞癌高分化细胞系 Ec9706、Ec109、TE1 进行常规细胞培养，我们从 mRNA 和蛋白水平方面，采取 Real Time PCR 和 Western blot 检测这 3 种细胞系中 MMP-16 的表达，并筛出相对高表达 MMP-16 的细胞系。
3. 设计合成 4 对 MMP-16 基因的 shRNA(shRNA-1, 2, 3,4)，采用阳离子聚合物试剂稳定转染 Ec109, 利用 Real Time PCR 和 Western blot 检测稳转 shRNA 后 MMP-16 mRNA 及蛋白的沉默效果，并筛选出合适的转染条件和干扰效率最高的 shRNA，以利于进一步的实验研究。
4. 取干扰效率最高的 shRNA，应用 WST-1 及流式细胞仪检测 MMP-16 基因表达下调后细胞增殖及凋亡情况；应用细胞划痕实验检测 MMP-16 基因表达下调后食管癌细胞迁移能力的变化；应用 Transwell 细胞侵袭实验检测 MMP-16 基因表达下调后，食管癌细胞 Ec109 侵袭能力的改变。

结果

1. MMP-16 蛋白在食管鳞状细胞癌组织标本中的低表达,在配对的远癌组织高表达,差异具有统计学意义 ($P<0.05$),MMP-16 蛋白在 T1/T2 期中的表达明显高于 T3/T4 期中的表达,差异具有统计学意义。MMP-16 蛋白的表达与食管鳞癌组织分化程度正相关 ($P<0.05$)。

2. 3 种食管鳞癌高分化细胞株均有 MMP-16 基因表达,其中 MMP-16 在 Ec109 细胞系中表达明显高于其它 2 种食管癌细胞株,差异具有统计学意义 ($P<0.05$);

3. 4 组 shRNA 稳转组 MMP-16 基因在 mRNA 和蛋白水平上均有显著沉默效果,基因沉默效果分别为 shRNA-1(89%)、shRNA-2(96%)、shRNA-3(80%)、shRNA-4(92%)。其中 shRNA-2 干扰效率最高,与空白对照及阴性对照组比较,差异具有显著性意义($P<0.01$);

4. 转染 shRNA 下调食管癌细胞 MMP-16 基因表达后,WST-1 检测 shRNA-2 干扰组与对照组增殖能力无显著性差异;流式细胞仪检测 shRNA-2 干扰组与对照组凋亡能力明显减退,有显著性差异 ($P<0.05$);细胞划痕 72h 后 shRNA-2 转染组细胞划痕明显愈合,而阴性对照组愈合较少,差异有统计学意义($P<0.05$);Transwell 小室细胞侵袭实验结果 shRNA 转染组穿膜细胞数明显增多,与阴性对照组比较,差异有显著性($P<0.05$)。

结论

1.首次从基因和蛋白水平上证实了 MMP-16 在临床组织标本食管鳞状细胞癌中的低表达,在配对的远癌组织高表达,随着肿瘤浸润深度的加深而 MMP-16 蛋白的表达减少,随着肿瘤分化程度的降低而 MMP-16 蛋白的含量减少。

2. 食管癌 Ec109 为 MMP-16 高表达细胞系,可作为进一步研究的实验材料。

3. 利用针对 MMP-16 基因的 RNA 干扰质粒转染食管癌细胞,可以有效下调 Ec109 细胞内 MMP-16 基因表达水平,可作为一种基因策略进行深入研究。

4. 从体外细胞实验,首次研究并证实了 shRNA 转染组与阴性对照组相比,细胞的增殖并没有显著变化,凋亡能力明显减弱,细胞迁移及侵袭能力水平均增加,推测 MMP-16 在食管鳞形细胞癌凋亡、迁移及侵袭中起着非常重要的作

用；MMP-16 将来可能在抑制食管鳞癌肿瘤的发生发展中起重要作用，MMP-16 将来可能会成为食管癌基因治疗的新靶点。

关键词：食管癌；短发夹 RNA；基质金属蛋白酶 16

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Abstract

Background and Object:

Esophageal carcinoma is one of main malignancies with rapidly developing and a poor prognosis in China, most are esophageal squamous cell carcinomas (ESCC). ESCC can hardly be detected in early esophageal cancer. So the insight into early detection of ESCC and the inhibition of ESCC cell progression are important. MMPs play essential roles in promoting tumor invasion and migration. Among all of the MMPs discovered recently. The recent studies have demonstrated that MMP-16 over expresses in some kinds of malignant tumor, and MMP-16 associated with some tumors invasion and poor prognosis. Now an experimental tissue inhibitor of metalloproteinase has been shown to little effect on tumor growth, it may even help tumor growth. the expression and functional significance of MMP-16 in esophageal squamous cell carcinoma development is remains unknown. RNA interference (RNAi) is the process of sequence specific post transcriptional gene silencing (PTGS) triggered by double stranded RNA (dsRNA). Now, RNAi is very useful technique and has been applied in gene function studies. In this study, we suppressed MMP-16 expression in esophageal carcinoma cell line Ec109 with RNA interference and then observed the behavior of Ec109 in invasion and migration after MMP16 gene expression was silenced.

Methods:

1. The expression of MMP-16 mRNA and protein in ESCC and the matched normal tissues (36 cases) was determined by immunohistochemistry, western blot and Real Time PCR.
2. Three esophageal carcinoma cell lines (Ec9706, Ec109 and TE1) were cultured using common methods. Western blot and real-time quantitative PCR were used to verify the expression level of MMP-16. The relationship between MMP-16 expression level and the malignant degree of esophageal carcinoma cell was analyzed. The MMP-16 over-expressed cell line was selected.
3. Four MMP-16 targeting shRNAs were designed and synthesized. These shRNAs were transiently transfected into Ec109 via cationic liposome sofastTM. Western blot and real-time quantitative PCR were used to verify the interference

efficiency of MMP-16 protein and mRNA expression levels. The shRNA with high interference efficiency and the eligible shRNA density were selected.

4. After MMP-16 was down-regulated in esophageal carcinoma cell line Ec109 shRNA, the ability of proliferation was studied by WST-1; The ability of apoptosis was researched by flow cytometry; the ability of cell invasion was measured by Transwell; the ability of cell migration was evaluated by wound healing assay.

Results:

1. MMP-16 protein was down regulated in cancerous group compared with the matched normal tissue and correlated with the clinical features of histological differentiation ($P<0.05$) and tumor stage ($P<0.05$). MMP-16 mRNA by Real Time-PCR showed that the level was no significant different in ESCC tissue compared with the matched normal tissue and little correlated with the clinical features.

2. MMP-16 expressed in all 3 esophageal carcinoma cell lines. The MMP-16 was significantly expressed in Ec109 as compared to the other cell lines ($P<0.05$).

3. The levels MMP-16 mRNA and protein in Ec109 were significantly decreased by RNA interference ($P<0.05$). The shRNA-2 is the most effective shRNA.

4. After MMP-16 expression down-regulated in Ec109 by shRNA, the ability of cell proliferation was no change; Cell apoptosis was inhibited ($P<0.05$); Cell invasion and migration ability were significantly promoted ($P<0.05$).

Conclusion:

1. MMP-16 is essential to the growth and development of high differentiated and moderately differentiated esophageal squamous cell carcinoma and esophageal epithelial tissue.

2. The MMP-16 over expression cell line, Ec109 is an ideal material for follow-up research.

3. ShRNA can efficiently down-regulate the MMP-16 gene in esophageal carcinoma cell line Ec109, shRNA should be a method for gene therapy.

4. MMP-16 plays an important role in the promotion of tumor apoptosis, the ability of migration and invasion of esophageal carcinoma cell line Ec109 can be promoted by MMP-16 silencing. MMP-16 may serve as a tumor protector in human ESCC. MMP-16 may be considered as a target gene for therapy of esophageal

carcinoma.

Key Words: Esophageal carcinoma; ShRNA; MMP-16

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主要缩略语表

英文简称	英文全称	中文全称
MMP16	Matrixmetall proteinase 16	基质金属蛋白酶 16
ESCC	Esophagus squamous cell carcinoma	食管鳞状细胞癌
VEGF	Vascular endothelial growth factor	血管内皮生长因子
TNF- α	Tumor Necrosis Factor-alpha	肿瘤坏死因子- α
MMPs	Matrix metalloproteinases	基质金属蛋白酶类
MMP-2	Matrix metalloproteinase-2	基质金属蛋白酶-2
MMP-9	Matrix metalloproteinase-9	基质金属蛋白酶-9
TIMP	Tissue inhibitor of metalloproteinase	金属蛋白酶组织抑制剂
ECM	Extracellular matrix	细胞外基质
GAPDH	Glyceraldehyde phosphate dehydrogenase	磷酸甘油醛脱氢酶
PCNA	Proliferating cell nuclear antigen	增殖细胞核抗原
TGF- β	Transforming growth factor beta	转化生长因子- β
LPS	Lipopolysaccharide	脂多糖
PBS	Phosphate Buffered Saline	磷酸盐缓冲液
DMSO	Dimethyl sulfoxide	二甲亚砜
BSA	Bovine serum albumin	牛血清白蛋白
FBS	Fetal bovine serum	胎牛血清
EB	Ethidium bromide	溴乙锭
PI	Propidine iodide	碘化丙啶
SDS	Sodium lauryl sulfate	十二烷基硫酸钠
ELISA	Enzyme-linked immunosorbent assays	酶联免疫吸附试验

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